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Soluble HLA class I antigens in serum of healthy individuals – population study

Gabriel Turowski, Anna Kędzierska

Laboratory of Clinical Immunology, Faculty of Medicine, Jagiellonian University, Cracow, Poland

SUMMARY

Variability of concentrations of s-HLA-I depending on allelic specificity substantiated realisation of research in population of 248 healthy, unrelated individuals. Defined phenotypes from tissue typing of polymorphic HLA complex enabled concentrations measurements of 1553 serum samples for HLA - A, B, and C loci determined antigens. Semi-quantitative technique of inhibition microcytotoxic reaction according to Tait et al. (1981) and Mclean et al. (1983) with usage of policlonal sera anti-HLA was applied.
For most of numbers of the allelic specificity the concentration of antigen material in soluble form (s-HLA-I) in blood serum were nominally very high and high. For certain numbers of specificity e.g. HLA- A26, A29, B39, B52, B56, Cw5, Cw6 the percentage of sera, where the s-HLA concentrations were decreased was observed. The results were presented as mean values of inhibition microcytotoxic reaction – according to NIH classification.
Authors point on usefulness of results for s-HLA comparative analysis of particular HLA allelic specificity, specific for certain diseases e.g. Cw7 for SNHL patients and B27 for ankylosing spongilitis

INTRODUCTION

The role of the class I HLA molecule in soluble form (s-HLA-I) in all life forms is substantial. Polymorphic sHLA molecules measured in blood examinations are similar to HLA antigens placed in membranes of various cells and tissues. s-HLA molecules have the same, basic structure and ability to bind with anti-HLA. s-HLA antigens form complexes with surface markers of molecules with CD4 and CD8 [1,2] respectively. Such fact indicates function of s-HLA molecules in regulatory mechanisms. Another function attributed to the s-HLA antigens is an ability to bind, through protein receptors, various endogenous and exogenous peptides [3].

Many years of polymorphic human histocompatibility complex research unquestionably proved linkage disequilibrium of individuals in population's certain disorders. Such stated problem proves that the research over confirmatory correlation on the level of soluble HLA antigens is needed.

Recently s-HLA function in various diseases through quantitative concentration fluctuations in serum blood samples of examined patients was evaluated. Puppo et al. [4] were observing correlation between s-HLA-I concentration and AIDS progress. According to their research s-HLA molecules in HIV-seropositive patients might have an influence on immunodeficiency pathogenesis. Similarly, Hagihara et al. [5] established significantly increased class I s-HLA and sCD8 blood serum concentration straight after and during interferon therapy in HCV-positive patients. According to authors, interferon may induce s-HLA-I production.

Results of our research suggested correlation s-HLA concentration with serum examinations of ankylosing spongilitis, sensory-neural deafness, head and brain injury patients [6,7,8].

Determination of soluble HLA-A2 and HLA-B7 presence was begun in 1970 by Van Rood and also

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Correspondence address: Gabriel Turowski, Laboratory of Clinical Immunology, Faculty of Medicine, Jagiellonian University, ul. Grzegórzecka 16, 31-531 Cracow, Poland

by Charlton and Żmijewski [9, 10]. Further research was continued by Tait et al. [11], McLean et al. [12] and also by Batko-Flasza and Turowski [13] with application of the s-HLA-I measurement method depending on the inhibition lymphocytotoxic reaction, independently from ELISA method, with application of monoclonal antibodies for s–HLA-I molecules.

The s-HLA concentration fluctuations for particular specificity of HLA class I in comparison of healthy and sick individuals seems to be interesting. The number of letters published in this matter is unsatisfactory. We decided to start evaluation of s-HLA-I concentration research in blood serum of healthy individual's population for particular antigens determined by HLA-A, HLA-B, HLA-C loci. The research was possible only with usage of cytotoxic anti-HLA antibodies for class I HLA antigens with application of the inhibition lymphocytotoxic reaction. Measurement's performance substantiated comparative analysis of s-HLA-I concentrations in blood serum of healthy and sick individuals.

MATERIAL AND METHODS

From venous blood examinations blood serum from 248 healthy individuals with defined HLA-I phenotypes was separated. In three serum dilutions, as the source of antigen material in soluble form, s-HLA concentration was measured. According to the s-HLA polymorphism and after taking into consideration homozygotic patterns and HLA blanc we obtained 1553 samples for multiple s-HLA concentration measurements.

Concentrations of soluble locus A determined antigens were evaluated in 446 samples, locus B in 787 and locus C in 320 samples. s-HLA concentrations were measured in semi-quantitative microabsorption test in inhibition lymphocytotoxic reaction according to McLean et al. [12] in the following, our own modification [13].

To each 1 μl of anti-HLA adequate specificity serum and with predetermined titre various amounts e.g. 0.5, 0.75, and 1 μl of examined individual serum as s-HLA source were added. Krebs-Ringer buffer solution was added to fulfil to 1 μl volume. After 3 hours of 20-22°C incubation the donor lymphocyte suspension with adequate phenotype was added and further step was analogous with microcytotoxity tissue typing test [14]. The control reacting reference system was composed of 1 μl of anti-HLA serum for tested phenotype and 1 μl of 3% human serum albumin (HSA) with 8 points in cytotoxic reactive force scale by NIH approved convention [14].

Inhibition cytotoxic reaction proved presence of the antigen material in soluble form (s-HLA) in serum was evaluated from 1 to 8 point scale. When there was no inhibition reaction, point values were high and the sum (Σ) for 3 dilutions of examined serum amounted in total 24 points, similarly to the control system. Three ranges of lymphocytotoxity reaction inhibition was assumed e. g. very high s-HLA concentration level (1–5 points), high concentration level (6–10 points), and low concentration level (11–24 points). According to the sum (Σ) inhibition lymphocytotoxic reaction of particular dilutions A relative value according to the $A=1/\Sigma_i \ldots . x100$ formula was calculated. Results were formulated in mean values with standard deviation and with percentage for number of positive serum of examined individuals.

RESULTS

From s-HLA-A concentration's distribution presented in table 1 comes conclusion that in blood serum of healthy individuals with present phenotype HLA-A68 [28], A24 [9] and A32 [19] antigens s–HLA concentration reached highest values. High concentration for HLA-A23 [9], A29 [19], A30 [19] and in single case A31 [19] specificity was not observed. In summary, from 446 examined samples in 198 (44.4%) very high s-HLA concentration values was measured, in 173 (38.8%) high s-HLA concentration values was detected. Considering percentage of serum samples for two s-HLA concentration ranges, worth mentioning is the fact that only in 57% of serum samples concerned s-HLA-A26 [10], 67% s-HLA-A29 [19], 74% s-HLA-A2 and 78% s-HLA–A31 [19]. For the rest of the antigens percentage fluctuated from 83 to 100. In table 1 inhibition lymphocytotoxic reaction results and A relative value for single serum samples were presented, in respect to the fact that the number of specificity including HLA-A19 is manifested with low frequency in our population.

The results of s-HLA concentration values for 23 allelic specificity B locus determined and for biallelic Bw4/Bw6 system were presented in table 2. From 292 examined samples very high s-HLA concentration values were observed in almost all soluble HLA-B antigens excluding HLA-B39 [6], B52 [5] and B56 [22]. Mean values of inhibition cytotoxic reaction sum and responding it A relative value for

Table 1. sHLA level in sera samples of healthy, adult persons.

sHLA-A	N	%	n	Very high				%	n	High				%	n	Low			
				∑		A				∑		A				∑		A	
				x	±SD	x	±SD			x	±SD	x	±SD			x	±SD	x	±SD
A1	61	33	20	3.5	1.3	34.9	19.3	49	30	7.1	1.2	14.4	2.2	18	11	12.0	1.6	8.5	0.9
A2	131	37	48	3.3	1.5	40.9	27.1	37	48	7.9	1.4	13.1	2.4	26	35	14.0	2.7	7.4	1.4
A3	78	33	26	3.6	1.2	31.2	18.0	55	43	7.7	1.5	13.5	2.5	12	9	11.9	1.3	8.5	0.8
A11	25	64	16	3.4	1.3	37.3	25.7	36	9	6.8	1.1	15.1	2.1	–	–				
A23(9)	13	–	–					85	11	8.6	1.0	11.9	1.8	15	2	11.0	–	9.1	–
A24(9)	40	95	38	2.1	1.4	66.9	34.4	2.5	1	7.0	–	14.3	–	2.5	1	23.0	–	4.4	–
A25(10)	20	45	9	3.4	0.9	30.9	8.7	50	10	7.9	1.5	13.1	2.5	5	1	12.0	–	8.3	–
A26(10)	28	32	9	3.9	1.5	34.3	26.6	25	7	8.6	1.3	11.9	1.8	43	12	16	2.3	6.4	0.9
A29(19)	3	–	–					67	2	9.0	–	11.1	–	33	1	12.0	–	8.3	–
A30(19)	6	–	–					83	5	7.8	1.5	13.2	2.5	17	1	18.0	–	5.6	–
A31(19)	9	11	1	5.0	–	20.0	–	67	6	8.0	1.6	12.9	2.6	22	2	15.5	2.1	6.5	–
A32(19)	17	94	16	2.4	1.1	50.7	26.3	6	1	6.0	–	16.7	–	–	–				
A68(28)	15	100	15	2.3	1.4	64.1	35.7	–	–					–	–				
Total	446	44.4	198					38.8	173					16.8	75				

∑ - sum of inhibition cytotoxic reaction; A - relative value; N - number of sera samples; n - number of sera samples in the range of cytotoxic reaction;
% - percentage of positive sera

particular tested serum dilutions, reached highest values for 21 serum samples of antigen s-HLA-B51 [5] and s-HLA-B58 [17] which appeared in only one phenotype. Similar concentration values were observed for s-HLA-B35, B41, B49 [21] and Bw6. In total, very high s-HLA concentration values for HLA-B antigens were evaluated in 292 (37.1%) serum samples from 787 examined. In the table 2 second column presented high s-HLA-B concentration results with standard deviation were close to each other due to the former inhibition cytotoxic reaction ranges selection. High concentration value of particular s-HLA-B antigens was evaluated in 42.6% serum samples. In total, s-HLA concentration value in 787 serum samples was high – 79.7%. Low s-HLA-B concentration values were detected only in 160 (20.3%) serum samples of all examined individuals.

In Table 3, in analogous way as above, research results of s-HLA concentration for 7 numbers of serologically typing locus C specificity were compiled. Very high s-HLA-C concentration values were evaluated in 101 serum samples (31.6%). Mean s-HLA concentration value was highest in reference to soluble HLA-Cw4 and Cw7 antigens with A relative value amounted 41.0±21.8 and 43.7±27.5 respectively. In 43.8% of the samples the s-HLA-C concentration value was high. Worth attention is result of s-HLA-Cw2 and s-HLA-Cw3 concentration values, where the percentage for high and very high concentration values range amounted 93 and 95. Low s-HLA concentration value was evaluated in 67% and 52% in HLA-Cw5 and HLA-Cw6 serum antigen phenotype of examined individuals. In 320 tested samples with C locus determined specificity only 22.8% evaluated low s-HLA-C concentration values.

DISCUSSION

In antigen concentration material research in soluble form (s-HLA-I) in blood serum samples of individuals not loaded with any pathological process, was unequivocally proven that concentration values of most tested s-HLA-ABC antigens were high. Only for particular number of specificity e.g. s–HLA-A26 [10], A29 [19], B39 [16], B52 [5], B56 [22], Cw5 and Cw6 some percentage of sera with low concentration values were observed. Observations mentioned above seem to prove the results of the conducted independently from our work, that in sera of all healthy individuals s-HLA class I are present. Low concentration values may be the consequence of subclinical pathological process in persons treated as 'healthy'.

The microabsorption in inhibition lymphocytotoxic reaction as s-HLA determination test is a relatively simple method, which enables s-HLA determination for soluble HLA class I polymorphic system specificity present in serum. Usage of inhibition cytotoxic reaction was started by Van Rood et al. [9] and also Charlton and Żmijewski [10] in 1970

Table 2. sHLA-B antigens level in samples of healthy, adult person.

sHLA-B	N	%	n	Very high				%	n	High				%	n	Low			
				∑		A				∑		A				∑		A	
				x	±SD	x	±SD			x	±SD	x	±SD			x	±SD	x	±SD
B7	60	15	9	4.4	0.7	23.2	4.5	60	36	8.1	1.3	12.7	2.2	25	15	12.9	2.1	7.9	1.0
B8	41	44	18	3.9	1.4	31.5	20.5	51	21	7.5	1.4	13.7	2.3	5	2	13.0	1.4	7.7	0.8
B13	22	45	10	3.4	1.1	32.3	10.8	32	7	7.1	1.2	14.3	2.4	23	5	15.4	2.8	6.6	1.1
B14	7	29	2	3.5	0.7	29.1	5.9	57	4	8.0	1.8	13.0	3.0	14	1	19.0	–	5.3	–
B18	40	52	21	3.6	0.8	29.1	7.0	28	11	8.4	1.6	12.4	2.6	20	8	15.3	3.1	6.8	1.3
B27	36	14	5	3.8	0.8	27.3	5.8	39	14	8.2	1.7	12.7	2.8	47	17	13.8	2.8	7.5	1.3
B35	35	63	22	2.8	1.2	46.4	27.3	31	11	7.9	1.2	12.9	2.2	6	2	12.0	1.4	8.4	0.9
B37	3	100	3	3.3	0.6	30.6	4.8	–	–					–	–				
B38(16)	16	50	8	3.6	1.3	31.5	12.7	44	7	7.1	1.7	14.6	2.9	6	1	15.0	–	6.7	–
B39(16)	6	–	–					67	4	9.8	0.5	10.3	0.6	33	2	14.0	0.6	7.1	0.8
B41	12	67	8	2.8	1.4	49.4	32.4	25	3	8.0	1.0	12.6	1.6	8	1	12.0	–	8.3	–
B44(12)	51	37	19	3.3	1.2	35.4	18.4	22	11	7.1	1.4	14.5	2.4	41	21	14.4	2.8	7.2	1.2
B49(21)	6	50	3	3.0	2.0	51.1	42.9	17	1	6.0	–	16.7	–	33	2	17.5	9.2	6.6	3.5
B50(21)	4	75	3	3.3	2.1	48.3	44.8	25	1	7.0	–	14.3	–	–	–				
B51(5)	23	91	21	1.2	0.5	92.1	20.1	9	2	6.0	–	16.7	–	–	–				
B52(5)	6	–	–					67	4	7.8	1.7	13.4	2.8	33	2	11.5	0.7	8.7	0.5
B55(22)	7	14	1	3.0	–	33.3	–	57	4	7.8	1.5	13.3	2.7	29	2	13.5	3.5	7.7	2.0
B56(22)	3	–	–					100	3	9.0	–	11.1	–	–	–				
B57(17)	16	38	6	4.0	1.1	27.5	11.3	50	8	7.1	0.9	14.3	1.9	13	2	12.5	0.7	8.0	0.5
B58(17)	1	100	1	1.0	–	100.0	–	–	–					–	–				
B60(40)	17	6	1	5.0	–	20.0	–	71	12	8.0	1.1	12.7	1.9	23	4	11.5	0.6	8.7	0.4
B61(40)	5	20	5	5.0	–	20.0	–	80	4	7.8	1.7	13.4	2.8	–	–				
B62(15)	23	52	12	3.3	1.3	35.6	13.5	35	8	6.3	0.5	15.9	1.2	13	3	13.0	1.0	7.7	0.6
Bw4	149	30	44	3.7	1.3	33.5	20.7	48	71	8.2	1.5	12.7	2.5	22	34	15.2	3.0	6.8	1.2
Bw6	198	37	74	2.9	1.3	46.1	27.7	45	88	7.8	1.4	13.2	2.4	18	36	13.7	2.4	7.5	1.2
Total	787	37.1	292					42.6	335					20.3	160				

∑ - sum of inhibition cytotoxic reaction; A - relative value; N - number of sera samples; n - number of sera samples in the range of cytotoxic reaction;
% - percentage of positive sera

and after them Tait et al. in 1981 [11] and McLean et al. in 1983 [12]. Published in 1994 Report of the Second International Soluble HLA (sHLA) workshop proves that inhibition cytotoxic reaction technique was applied independently from ELISA test in conditions, when highly specific monoclonal antibodies were used for this method designations [15]. In 1993 Pouletty et al. [16] in sHLA-B27 designation in 151 blood serum samples established that both methods compatibility reached 99.2%. Application of one monoclonal serum, anti W6/32 most often, for ELISA test created a question for how much this testing is going to show s-HLA concentration value deficit in examined body fluid material. Results of both techniques will be compared in future. Rubens et al. [17], Westhoff et al. [18], and Drouet et al. [19] during testing various molecular s-HLA class I variants pointed on its various biologic role. According to authors mentioned above serum soluble HLA antigens create immunological complexes with anti-HLA autoantibodies, contributing to autotolerance preservation. Additional role of soluble HLA class I antigen in immunity regulation was annotated by Saririan et al. [20] pointing on s-HLA-I concentration values correlation in serum before vaccination with response to influenza vaccination. According to recent paper publication possible immunoregulatory function of s-HLA class I molecules in NK cells identification was suggested. In such context various NK cells clones specifically identify various s–HLA antigens [21].

In accepted by our team in 1995 hypothesis about coincidence of s-HLA-B27 in pathological mechanism of ankylosing spongilitis and relation between s–HLA-B27 blood serum concentration values and clinical status of ankylosing spongilitis patients. Decrease of s-HLA-B27 blood serum concentration values of sick patients may occur

Table 3. sHLA-C antigens level in sera samples of healthy, adult person.

sHLA-C	N	%	n	Very high				%	n	High				%	n	Low			
				∑		A				∑		A				∑		A	
				x	±SD	x	±SD			x	±SD	x	±SD			x	±SD	x	±SD
Cw1	16	19	3	4.7	0.6	21.7	2.9	56	9	7.9	1.4	13.0	2.4	25	4	12.3	1.3	8.2	0.8
Cw2	44	32	14	4.1	0.7	25.4	4.9	61	27	7.5	1.3	13.7	2.3	7	3	11.3	0.6	8.8	0.4
Cw3	38	37	14	3.9	1.1	28.6	10.4	58	22	7.5	1.7	14.0	2.9	5	2	11.0	–	9.1	–
Cw4	63	35	22	2.9	1.2	41.0	21.8	35	22	7.7	1.4	13.4	2.4	30	19	14.2	2.7	7.3	1.2
Cw5	18	11	2	4.0	–	25.0	–	22	4	8.0	2.3	13.3	3.9	67	12	13.2	1.9	7.7	1.0
Cw6	40	10	4	4.3	0.5	23.8	2.5	38	15	8.4	1.5	12.3	2.5	52	21	13.0	2.1	7.8	1.1
Cw7	101	42	42	3.1	1.4	43.7	27.5	41	41	7.5	1.4	13.7	2.5	17	18	14.7	4.2	7.2	1.7
Total	320	31.6	101					43.8	140					22.8	73				

∑ - sum of inhibition cytotoxic reaction; A - relative value; N - number of sera samples; n - number of sera samples in the range of cytotoxic reaction;
% - percentage of positive sera

due to consumption or synthesis disturbances that's why purposefulness of s-HLA-B27substitution through serum transfusion of phenotype HLA-B27$^+$ blood donors [6].

Zavazava et al. [22] demonstrated low s-HLA concentration values for A1 antigens in homozigotic set, A26 and B40. Drouet et al. [23] stated that s–HLA antigens is present in serum of healthy individuals with wide range of concentrations. Mean s–HLA-B and s-HLA-C concentration value was phenotype dependent, e.g. statistically substantially higher, if in tested phenotype antigens HLA-A29 [19] and HLA-B44 were present. Results of so far performed research point that physiological consequences of s-HLA molecules has not yet been fully explained and they are still intriguing phenomenon in human life. Relatively small number of s-HLA presence publications substantiated conduction of certain tests in polish population of healthy individuals. Results are helpful for comparative analysis of s-HLA concentrations in sera of healthy and sick individuals, particularly in disorders, where s-HLA molecules participation in organism's metabolism is evident.

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